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Mitochondrial ATP-sensitive potassium channel activity and hypoxic preconditioning are independent of an inwardly rectifying potassium channel subunit in *Caenorhabditis elegans*

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ABSTRACT

Hypoxic preconditioning (HP) is an evolutionarily-conserved mechanism that protects an organism against stress. The mitochondrial ATP-sensitive K⁺ channel (mK_{ATP}) plays an essential role in the protective signaling, but remains molecularly undefined. Several lines of evidence suggest that mK_{ATP} may arise from an inward rectifying K⁺ channel (Kir). The genetic model organism *Caenorhabditis elegans* exhibits HP and displays mK_{ATP} activity. Here, we investigate the tissue expression profile of the three *C. elegans* Kir genes and demonstrate that mutant strains where the *irk* genes have been deleted either individually or in combination can be protected by HP and exhibit robust mK_{ATP} channel activity in purified mitochondria. These data suggest that the mK_{ATP} in *C. elegans* does not arise from a Kir derived channel.

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1. Introduction

Brief periods of cell stress can activate endogenous protective mechanisms that reduce injury upon subsequent exposure to stress. Ischemic preconditioning (IPC) is one such example, whereby short periods of ischemia prior to the longer ischemic insult protect against ischemia-reperfusion (IR) injury. Since its discovery [1], IPC has been described in all species tested thus far including the genetic model organism *Caenorhabditis elegans*, where it has been termed hypoxic preconditioning (HP) [2]. Mitochondrial K⁺ channels play an important role in protection, and the activation of the mitochondrial ATP-sensitive K⁺ channel (mK_{ATP}) is necessary and sufficient to protect against IR injury [3,4]. Since the mK_{ATP} is a critical component of IPC, the channel has been the subject of intense investigation and has been found in humans [5], rats [6], plants [7], ameba [8], *Trypanosoma cruzi* [9], and in *C. elegans* [10].

Abbreviations: BTC-AM, benzothiazole coumarin acetyloxymethyl ester; IPC, ischemic preconditioning; mK_{ATP}, mitochondrial ATP-sensitive K⁺ channel; WT, wild-type; IRK (Kir), inwardly rectifying potassium channel subunit

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The molecular identity of mK_{ATP} remains elusive, but is thought to resemble the surface K_{ATP} channel, in which the pore forming subunit is a member of the inward rectifier potassium channel (Kir) family. Kir channels perform a diverse array of physiologic functions, and gene mutations have been associated with a variety of pathological conditions in humans [for review, see [11]]. The mammalian Kir gene family consists of fifteen isoforms divided into seven subfamilies (Kir1.x to Kir7.x). These subfamilies can be further classified into four functional groups, including classical, or constitutively active, G-protein gated, ATP-sensitive, and K⁺ transport channels. Surface K_{ATP} channels are composed of tissue specific combinations of inward rectifying K⁺ channel (Kir6.x) subunits and regulatory sulfonylurea receptor (SURx) subunits [12], and mK_{ATP} channels have been proposed to arise from some combination of these. In cardiac myocytes, the surface K_{ATP} and the mK_{ATP} resemble each other with respect to physiologic transport characteristics, but they can be distinguished by pharmacology. For example, diazoxide and 5-hydroxydecaonate (at appropriate concentrations) selectively activate and inhibit the cardiac mK_{ATP}, respectively [13]. These reagents, however, are not completely specific for the mK_{ATP}, as they can also regulate surface channel activity in other cell types, leading to

the idea that the pharmacologic dissimilarity between the surface and mitochondrial channels arises from some unique combination of Kir6.x and SURx, or novel splice variants [14]. Nevertheless, despite the tools available and its critical role in the evolutionarily conserved endogenous mechanism of IPC, the mK_{ATP} has not been conclusively defined at the molecular level using pharmacologic techniques.

The use of genetic models has also failed to result in the molecular identification of the mK_{ATP} . A mouse knockout model of Kir6.2 was shown to be resistant to IPC [15], but this resistance was not related to the loss of the mK_{ATP} . This may be linked to the fact that mutations in both Kir6.2 and SUR1 have both been linked to familial persistent hyperinsulinemic hypoglycemia [16], coupled with knowledge that hyperglycemia can abrogate protection by IPC [17]. Similarly, Kir6.1 and SUR2 knockout mice both display a phenotype indicative of Prinzmetal angina [18,19], complicating an analysis of its role in IPC. However, *C. elegans* lacks a vasculature and its genome codes for only three Kir family channels, *irk-1*, *irk-2*, and *irk-3*. Hence, we hypothesized that this genetic model, which can be protected by HP [2] and exhibits a mK_{ATP} activity [10] would be a useful reductionist model to assess the role of the Kir gene family in protective signaling.

Unlike mammals, the *C. elegans* family is limited to three genes, termed *irk-1*, *-2*, and *-3*. The reduced complexity of this family compared to that in humans would be expected to facilitate dissection of Kir function in worms. However, other than the fact that they are all most closely related to Kir2.x family members, with ~50% amino acid identities, there is very little known about them. Herein, we used fluorescent transgenes to probe the cellular expression pattern and protein localization of all three Kir genes (*irk-1*, *-2*, and *-3*) in *C. elegans*. Using worm genetic knockouts we determined if these genes were involved in HP or contributed to mK_{ATP} activity in isolated mitochondria. Our results suggest that the worm inwardly rectifying potassium channel subunit (IRK) channels have a limited distribution, which is inconsistent with a conserved function in mitochondria, and do not contribute to either HP or mK_{ATP} activity.

2. Materials and methods

2.1. *C. elegans*

Worm strains were routinely propagated at 20 °C on nematode growth media agar plates containing 5 µg/ml cholesterol and seeded with OP50 bacteria. Strains used in this study include Bristol-N2; fluorescent protein fusion strains (all in a *pha-1(e2123ts)III*, *him-5(e1490)V* genetic background) KWN73 rnyEx040 [pKT48 (*Pirk-1::IRK-1::GFP*); pCL1 (*pha-1+*)], KWN83 rnyEx043 [pKT54 (*Pirk-3::GFP*); pCL1 (*pha-1+*)], KWN88 rnyEx045 [pKT49 (*Pirk-2::IRK-2::GFP*); pCL1 (*pha-1+*)]; and *irk* gene deletion strains MT15934 *irk-1(n4895)X*, MT15935 *irk-2(n4896)X*, MT17369 *irk-3(n5049)X*, MT17360 *irk-1(n4895)X*, *irk-2(n4896)X*, *irk-3(n5049)X* (these mutants were generously provided by N. Ringstad and R. Horvitz). The triple deletion was obtained by a conventional mating strategy with PCR genotyping used to confirm the presence of the mutant alleles in the final strain.

2.2. Fluorescent imaging

Transgenic *C. elegans* strains expressing the fluorescent *irk* gene fusions were placed onto 2% M9 agarose pads under anesthesia (1 mg/ml tetramisole) and examined using an Olympus FV1000 confocal microscope (available as part of the University of Rochester Confocal Core) under appropriate illumination.

2.3. IR killing and HP of *C. elegans*

Briefly, *C. elegans* were chronologically-synchronized and adult hermaphrodites were collected, washed and allowed to lay embryos for a period of 2 h. The resulting adult progeny were subject to the following protocols as previously described [2]. **Simulated ischemia-reperfusion (IR) killing:** *C. elegans* in M9 were incubated at 26 °C for 20 h in an anoxic chamber (5%/95% H_2/N_2). Following the ischemic insult, *C. elegans* were returned to normoxia at 20 °C for 24 h. Animals were then scored. Animals which lack movement (spontaneous or touch-evoked) were scored as dead. **Hypoxic preconditioning (HP):** *C. elegans* were incubated for 4 h in an anoxic chamber at 26 °C. Following the stimulus, *C. elegans* were moved to normoxia at 20 °C for 20 h to allow for recovery. The preconditioned *C. elegans* were then subjected to IR killing as described above. For all experiments, seven replicates of at least thirty *C. elegans* were observed under paired IR and HP protocols for each of the mutant strains.

2.4. Isolation of mitochondria, BTC-AM loading, and mK_{ATP} thallium flux assay (Tl^{+} -flux)

Mitochondria were isolated from *C. elegans* as previously described [10]. The mitochondrial suspension was placed in a chilled stirring cuvette and incubated in the dark with 20 µM benzothiazole coumarin acetyoxymethyl ester (BTC-AM) (Invitrogen) and 0.05% Pluronic F-127 for 10 min at room temperature. Mitochondria were washed to remove excess BTC-AM by centrifugation at 7000×g for 10 min. The pellet was resuspended in MIM (220 mM mannitol, 70 mM sucrose, 5 mM MOPS, 2 mM EGTA, 0.4% BSA, pH 7.4, filtered), stored on ice in the dark and used within 1.5 h of isolation. Protein was determined by the Folin-phenol method [7].

mK_{ATP} activity was determined by monitoring the uptake of Tl^{+} , a surrogate for K^{+} , into mitochondria using a Varian Cary Eclipse spectrofluorometer as previously described [20]. Briefly, ~0.8 mg/ml BTC-AM-loaded mitochondria were added to a rapidly stirred cuvette containing 2 ml of Tl^{+} assay buffer (2 mM pyruvate, 2 mM malate, 195 mM mannitol, 10 mM HEPES, 2 mM $MgCl_2$, 2 mM Na_2HPO_4 , and 1 µg/ml oligomycin, pH 7.2 at 25 °C). Compounds were present in the buffer and incubated with mitochondria for 30 s. Tl_2SO_4 (2 mM) was added via a syringe port and the change in fluorescence was monitored (λ_{ex} 488 nm, λ_{em} 525 nm) and normalized to an initial baseline recording 10 s.

2.5. Reagents

Chemicals were of the highest grade available from Sigma (St. Louis, MO) unless otherwise specified. BTC-AM was from Invitrogen (Carlsbad, CA). Atpenin A5 was from Axxora LLC (San Diego, CA),

2.6. Statistics

In isolated mitochondrial studies, statistical significance ($P < 0.05$) between multiple groups was determined using analysis of variance (ANOVA). In whole worm studies, significance ($P < 0.05$) was determined using a paired Student's *t*-test. Data presented are mean ± S.E.M.

3. Results

3.1. Expression profiles of *irk* genes

C. elegans hermaphrodites are comprised of ~1000 somatic cells that share many basic cell biologic processes with mammals. In order to define the cells and cell types that express the *irk* genes,

each of the three *irk* promoters was amplified using genomic PCR and fused via PCR sewing to the coding region of GFP. These PCR products were used to create transgenic nematode strains where the fluorescent cells demarked the limits of *irk* gene expression (transcriptional reporters *Pirk-x::GFP*). As a complementary approach, the entire *irk* coding sequence was amplified together with its cognate promoter and fused in-frame to GFP (translational reporters *Pirk-x::IRK-x::GFP*). Translational reporters give rise to fluorescent IRK fusion proteins that can be used to determine intracellular protein localization. It should be noted when examining worms expressing these constructs that promoter-driven transgenes may not completely recapitulate endogenous expression patterns, either due to mosaicism (where individual worms may not exhibit the full repertoire of cells due to loss of the transgene during mitosis) or because sequences in the genomic region such as the 3' UTR contribute to proper expression.

Fig. 1 shows representative images of transgenic strains expressing fluorescent reporters for each of the *irk* genes. For the *irk-1* and *irk-2* genes, their respective transcriptional and translational fusions were expressed in a similar repertoire of

cells (data not shown), and hence only the translational fusions are shown. The *irk-1* promoter drove GFP expression in multiple neurons, including the hermaphrodite specific neuron (HSN), sensory phasmid neurons in the tail, and unidentified neurons in both the head and tail of male worms (Fig. 1A–D). Intestinal and gonadal cells were labeled as well (Fig. 1E–F). As would be predicted for all of the IRK proteins based upon homology with the mammalian Kir channels, clear plasma-membrane targeting of the GFP-tagged IRK-1 protein can be observed in the intestine (Fig. 1C and E). This does not preclude dual targeting of the protein to the mitochondria, however, and it should be noted that enforced expression of fluorescent fusion proteins in worms can lead to targeting artifacts. However, the *irk-1* cDNA expressed in *Xenopus* oocytes gave rise to a plasma membrane K^+ conductance (N. Ringstad, personal communication), suggesting that IRK-1 is a bona fide plasma membrane channel. The fusion protein was likely active, as well, since strains expressing it were viable but were not healthy, and exhibited signs of cellular degeneration over time. For example, the neuronal processes in adults assumed a tortuous morphology and the fusion proteins appeared to

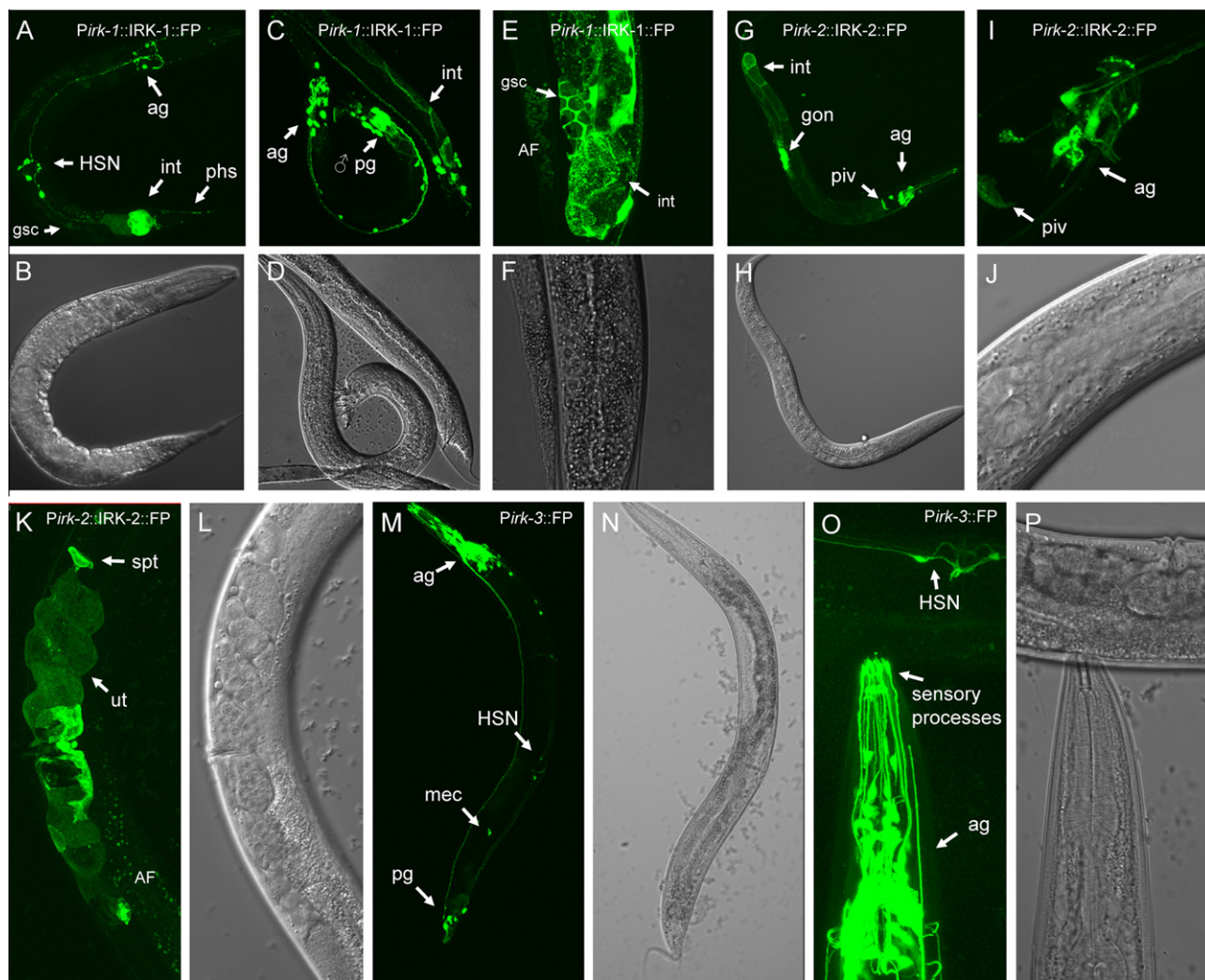


Fig. 1. Expression analysis of the *irk* genes in *C. elegans*. The promoter and genomic ORFs of *irk-1* (A–F) and *irk-2* (G–L) and the promoter of *irk-3* (M–P) were amplified via PCR, fused to the ORF of GFP, and micro-injected to create transgenic strains. Representative fluorescent confocal (A, C, E, G, I, K, M, and O) and Normarski (B, D, F, H, J, L, N, and P) images are shown indicating the cells where the promoter is active. The *irk-1* and *irk-2* translational fusions additionally demonstrate plasma membrane localization of the respective fusion proteins. The worms in panels C and D are male (♂). Abbreviations: ag, anterior ganglia; AF, intestinal autofluorescence; bwm, body wall muscle; gon, gonad; gsc, gonadal sheath cell; HSN, hermaphrodite specific neurons; int, intestine; mec, mechanosensory neurons; pg, posterior ganglia; phs, phasmid neurons; piv, pharyngeal intestinal valve cells; spt, spermatheca; ut, uterus.

aggregate over time (Fig. 1A). This can occur due to overexpression or misregulation of a “leak” conductance type channel that is normally only active under select conditions.

As for IRK-1, IRK-2 is also expected to be targeted to the plasma membrane. The most notable cells labeled by *irk-2* promoter expression were neurons of the anterior ganglia, the pharyngeal intestinal valve cell, and the posterior intestine, where again clear basolateral plasma membrane targeting can be observed (Fig. 1G–L). In adults, the spermatheca and the uterus were prominently labeled (Fig. 1K), and the IRK-2 fusion protein resided in the plasma membrane.

The *irk-3* translational fusion was not tolerated by the worms, even at low doses. Hence, a transcriptional fusion was used to assess the extent of *irk-3* gene expression. *Irk-3* promoter activity was limited to neurons, but it was broadly expressed throughout multiple neuronal subtypes, including sensory neurons and the HSN (Fig. 1M–O).

It is interesting that *irk* gene expression was notably absent in muscle and hypodermal cells. These cell types are amongst the largest in *C. elegans* and would be expected to contribute greatly to their total mitochondrial content. A channel that is localized to the mitochondria, even in part, and contributes to mitochondrial protection from insult might be predicted to be expressed more ubiquitously than observed here for the *irk* genes.

3.2. Hypoxic preconditioning in *C. elegans* is independent of *irk* genes

Since HP is widely conserved across species and is known to protect *C. elegans* from hypoxic death [2], and worms mitochondria exhibit robust mK_{ATP} channel activity [10], we sought to determine whether the *C. elegans* mK_{ATP} channel is comprised of a Kir family gene product. Mammals contain fifteen Kir genes, while *C. elegans* only contains three genes in the Kir family, termed *irk-1*, *irk-2*, and *irk-3*. As is often the case with large gene families, it is difficult to identify mammal–worm orthologs, and the worm genes are more closely related to one another than to any of the mammalian isoforms. The mammalian gene product most closely related to the worm *irk* family is Kir2.1. However, it should be noted that K_{ATP} channels open spontaneously in the absence of ATP and can be broadly classified with the classical Kir2.x channels which have constitutive activity.

C. elegans loss-of-function mutants in the *irk-1*, *irk-2*, and *irk-3* genes are viable, and due to the absence of tissues that are known to depend on K_{ATP} function in mammals (e.g. pancreatic beta-cells) in the worm, we hypothesized that they would be less likely to present confounding issues. First, we sought to determine if the *irk* gene products were required for HP. The worm HP model is a model of delayed preconditioning where a short 4 hr hypoxic exposure, followed by 20 h of normoxic recovery, induces resistance to a subsequent 24 h hypoxic killing. As shown in Fig. 2, HP significantly protected the wild-type (WT) strain from hypoxic stress, as previously demonstrated [2,21]. Unexpectedly, all of the *irk* deletion strains were also protected by HP to the same extent as WT, as was a triple deletion strain containing combined *irk-1*, *irk-2*, and *irk-3* mutant alleles (Fig. 2). However, the triple deletion strain exhibited somewhat less variability in its basal sensitivity to hypoxia than the WT and, though statistically insignificant, appeared slightly more resistant to hypoxia on average (WT, 60% death; triple, 50% death; *P*-value 0.30). In conclusion, these results suggest that HP in *C. elegans* is independent of a Kir family K^+ channel.

3.3. mK_{ATP} channel activity in *C. elegans* is independent of *irk* genes

We next sought to determine whether *C. elegans* mK_{ATP} activity was affected by the loss of *irk* gene function in the deletion

mutants. The mK_{ATP} contributes to K^+ transport across the mitochondrial inner membrane, and channel activity can be monitored by fluorescent measurements of Tl^+ fluxes (as a surrogate for K^+) in isolated mitochondria using the dye BTC-AM [20,21]. Using this technique, we measured changes in mitochondrial Tl^+ uptake in response to a conserved repertoire of activators and inhibitors that together define a pharmacologic signature for mK_{ATP} activity. We then compared this signature in WT worms to that in the *irk-1/-2/-3* triple deletion mutant to assess the contribution of worm *irk* genes to mK_{ATP} activity.

In WT worms we observed mitochondrial Tl^+ flux that was inhibited by ATP and activated by both diazoxide and atpenin A5 (Fig. 3). This activation by channel openers could be inhibited by mK_{ATP} blockers 5-hydroxydecanoate and glyburide (Fig. 3), consistent with mK_{ATP} activity in isolated *C. elegans* as well as mammalian mitochondria [10]. Mitochondria from the triple *irk* deletion strain exhibited a nearly identical profile, providing further evidence that mK_{ATP} is not derived from a Kir protein in *C. elegans*.

4. Discussion

The mK_{ATP} is central to IPC-mediated protection yet its molecular identity has remained elusive. The simplest hypothesis put forth thus far has been that the protein composition of the mK_{ATP} resembles that of the defined surface K_{ATP} , which is comprised of an octamer of four Kir channel subunits and four SUR accessory subunits. However, our results demonstrate that neither HP nor mK_{ATP} requires Kir gene function in the nematode *C. elegans*.

One interesting outcome of the current data is the possibility that Kir genes are also not involved in the mK_{ATP} in mammals. Significant effort has been directed toward assessing a role for putative K_{ATP} components (e.g. Kir6.2, Kir6.1 and SUR) in mammalian mitochondria, with ambiguous results. These efforts can be broadly categorized into three areas. The first of these relies upon antibody detection, the second on genetic elimination of candidates, and the third on pharmacologic manipulation of channel activity.

Approaches based primarily on antibody labeling concluded that either Kir6.2 [5], Kir6.1 [22–24], both [25–27] or neither [28] were present in mitochondria, perhaps due to variability in reagent quality. In this regard, two commercially available anti-Kir6.1 antibodies were shown to recognize off-target mitochondrial proteins, NADH-dehydrogenase flavoprotein 1 and isocitrate dehydrogenase [29]. Using custom antibodies, a novel splice variant of SUR2 has been identified in cardiac mitochondria [14], but its relevance to mK_{ATP} is as of yet unclear.

To attempt to circumvent this type of confusion, genetic loss-of-function mouse models have been developed for Kir6.1 [19], Kir6.2 [16], SUR1 [30], and SUR2 [31]. However, these models exhibited either incomplete loss of gene activity [32], or confounding effects (e.g. elevated blood glucose) that prevented unambiguous interpretation of their ability to be effectively protected by IPC [16–19]. Despite this, a technique utilizing flavoprotein fluorescence has been employed as an indirect measurement of mK_{ATP} activity in genetic deletion or dominant negative gene transfer models, and neither Kir6.1 nor Kir6.2 has been found to play an important role in mK_{ATP} opening-induced flavoprotein fluorescence [15,33]. This result is in general agreement with our finding that none of the Kir genes plays a role in mK_{ATP} activity in worms. Furthermore, the mK_{ATP} has been described in plants recently, where the single Kir gene was hypothesized to be unlikely to contribute to the formation of the mK_{ATP} [34]. Clearly, genetic deletion of all 15 Kir isoforms in mammals is not practical, but these results do suggest that the search for the mK_{ATP} should explore other non-Kir candidates.

Pharmacologic approaches to studying mK_{ATP} have been similarly ineffective at channel identification. In general, compounds

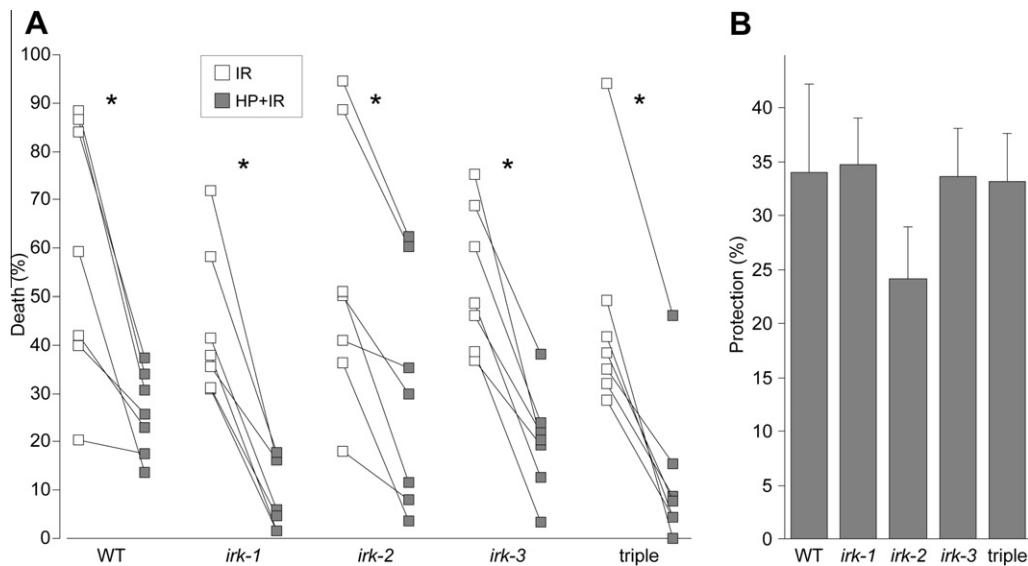


Fig. 2. HP in *C. elegans* is independent of the Kir family gene products *irk-1*, *-2*, and *-3*. (A) *C. elegans* WT control (N2-Bristol), and mutant deletion strains containing either the *irk-1*(n4895)X, *irk-2*(n4896)X, or *irk-3*(n5049)X alleles or all three mutant alleles in combination (triple) were subjected to simulated ischemia reperfusion (IR; white squares) or hypoxic preconditioning plus IR (HP+IR, gray squares) as detailed in the methods. Viability is expressed as percent of dead worms. To minimize handling artifacts, paired trials were performed on the same day using worms of identical age, and the IR and HP+IR values for each paired trial are linked together in the plot. (B) In order to compare different mutants, the degree of protection afforded by HP (equivalent to the IR minus the HP+IR for each paired trial) was averaged across the seven trials for each genotype. Means \pm S.E.M., $N = 7$, (N = independent trials performed on separate days, with each trial consisting of the average value obtained from 3 plates of worms, with >50 worms per plate), * $P < 0.05$ vs. IR.

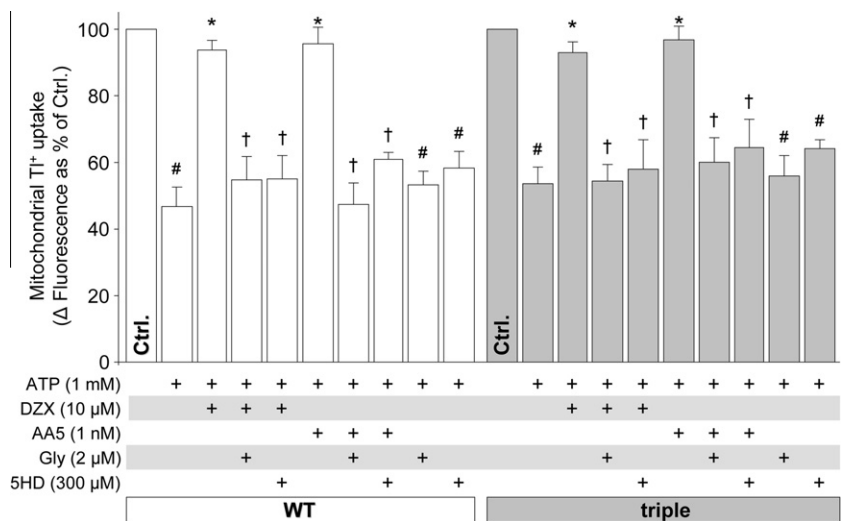


Fig. 3. *C. elegans* mK_{ATP} channel activity is independent of the Kir family gene products *irk-1*, *-2*, and *-3*. *C. elegans* mK_{ATP} channel activity in mitochondrial purified from N2-Bristol control worms (WT; white bars) or the *irk-1*(n4895);*irk-2*(n4896);*irk-3*(n5049) triple mutant (triple; gray bars). The baseline Δ fluorescence (Ctrl, set to 100%) was 15.3 ± 3.0 and 15.8 ± 2.7 arbitrary units for the WT and triple mutant, respectively. Pharmacologic agents were present as listed below the x-axis. Abbreviations: AA5, Atpenin A5; DZX, diazoxide; Gly, Glyburide; 5HD, 5-hydroxydecanoate. Data are means \pm S.E.M., $N > 4$ (N = independent mitochondria preparations). # $P < 0.05$ vs. Ctrl., * $P < 0.05$ vs. ATP, † $P < 0.05$ vs. ATP + DZX or ATP+AA5.

which open the surface K_{ATP} , activate the mK_{ATP} , and cause cardioprotection. Likewise, inhibitors of the surface K_{ATP} block the mK_{ATP} . This cross-reactivity suggests some degree of conservation between these two channels, but makes it difficult to study the role of either one in isolation. This fact along with other shortcomings has caused some to question whether a mK_{ATP} channel contributes to cardioprotection or exists at all [35]. However, there are a few pharmacologic tools that have used to dissect mK_{ATP} from the surface K_{ATP} . At low concentrations in cardiac myocytes, diazoxide and 5-HD are specific mK_{ATP} modulators and do not regulate the surface channel [13]. However, even in myocytes at higher

concentrations they have additional effects that might be predicted to impact IPC independent of their effect on mK_{ATP} [36,37]. These alternate targets may actually provide some insight into the molecular nature of the mK_{ATP} . For example, the specific activator of the mK_{ATP} , diazoxide, also inhibits complex II of the mitochondrial respiratory chain [36]. This characteristic is not unique to diazoxide, as the same result was observed for malonate [38] and atpenin A5 [39] amongst other complex II inhibitors [40]. Yet another study demonstrated that a purified mitochondrial inner membrane fraction displayed K^{+} channel activity that was sensitive to mK_{ATP} activators and

blockers but also responded to complex II inhibitors [28]. Hence, a moonlighting role for complex II in mitochondrial K⁺ transport has been hypothesized.

While the data presented herein demonstrate that a Kir isoform is not involved in the composition of the mK_{ATP}, they do not rule out the participation of a SUR subunit. In this regard, mK_{ATP} activity can be inhibited with the sulfonylurea glyburide (glybenclamide) even in the *irk* triple mutant (Fig. 3). It is possible that a SUR might associate with an alternate K⁺-transporting subunit to give rise to mK_{ATP} activity. It is also possible, as discussed for diazoxide, that glyburide may have an alternative target [41,42]. In this respect, glyburide has been shown to interact with mitochondrial ADP/ATP carriers [43]. Further work is undoubtedly necessary to elucidate the role of a SUR subunit in the composition of the mK_{ATP}.

Another interesting candidate arises from recent work examining the regulation of mK_{ATP} by redox status [40]. While a general trend of channel inhibition was observed with reducing agents of increasing redox potential, it was observed NADPH did not conform to this trend, i.e., despite identical redox potentials, NADPH was far more inhibitory to channel function than NADH. This suggests that NADPH is able to inhibit mK_{ATP} via a non-redox mechanism. Notably, the beta subunit of the “Shaker” family Kv channels has been shown to contain an NADPH binding site, coupled to an aldo-ketoreductase enzymatic activity [44,45]. In addition, single channel patch-clamp experiments from plant mitochondria are consistent with a Shaker like conductance, though the electrophysiologic characteristics of the single plant Kir gene product are unknown [34]. This raises the intriguing possibility that an NADPH binding Kv-beta subunit may be involved in mK_{ATP} assembly and/or regulation. Similarly intriguing perhaps is the observation that Kv1.3 forms a mitochondrial channel in lymphocytes that regulates apoptosis [46,47].

5. Conclusions

The quest for the molecular identity of mK_{ATP} channel remains. Antibody based approaches, pharmacologic regulatory strategies and mammalian genetic models have led to ambiguous conclusions and suffer from confounding effects. Furthermore, compensatory mechanisms involving other Kir subunits may rescue the mK_{ATP} activity in knockout animals. The data presented herein demonstrate effective HP and normal mK_{ATP} activity in *C. elegans* that do not have any of the three inwardly rectifying K⁺ channel genes. Furthermore, the use of a strain where all the Kir family genes in *C. elegans* have been knocked out together rules out the possibility of compensatory mechanisms involving another Kir isoform. These results suggest that the evolutionarily conserved phenomena of the mK_{ATP} and IPC may not center on a Kir component and should drive investigation of alternate targets.

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